REMARKS

The Pending Claims

Prior to entry of the above amendments, Claims 1-48 are pending. Claims 1-16 are directed to a method of transdifferentiating an epidermal basal cell into a cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal or glial cell. Claims 17-24 and 29-38 are directed to a transdifferentiated cell of epidermal origin having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal or glial cell. Claim 28 relates to a cell culture derived from the transdifferentiated cell of Claim 17, and Claim 39 relates to a cell culture derived from the transdifferentiated cell of Claim 29. Claim 40 relates to a method of using cells transdifferentiated from epidermal basal cells to identify a novel nerve growth factor. Claim 41 relates to a method of using cells transdifferentiated from epidermal basal cells to identify a potential chemotherapeutic agent. Claim 42 relates to a method of using cells transdifferentiated cells to screen a potential new drug to treat a nervous system disorder of genetic origin. Claims 43-48 are directed to a kit for transdifferentiating an epidermal basal cell into a cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal or glial cell.

The Office Action and Applicant's Amendment and Response

Applicant has amended the specification at page 1, line 4, to update the continuing data.

The amendment of Claim 1, which adds paragraph designations (a)-(c) is merely for greater clarity and convenience, and is not related to any issue of patentability.

The amendment of Claim 1, in the last paragraph (c) at line 11, which recites ". . . in an amount effective to suppress the expression of functional gene product of MSX1 and/or HES1 . . ." is merely for greater clarity, and is not made in response to any rejection or prior art of record. Support for the amendment is found, for example, in the same paragraph of Claim 1, at line 9; especially at page 10, lines 1-4; and in Table 1, page 29, lines 24-25.

The amendment of Claims 41 and 42, at first and second lines in step (d), to recite "chemotherapeutic agent" instead of "nerve growth factor" is to correct a typographical error. Support for the amendment is found, for example, in the preamble, step (c), and the last line of step (d) of each of Claims 41 and 42, as originally filed.

The Examiner provided an initialed Information Disclosure Statement, a Notice of References Cited, and a Notice of Draftsperson's Patent Drawing Review, which indicated that the Figures were declared informal.

Claims 1-48 were rejected for the following reasons.

A. Rejection of Claims 1-48 under the Doctrine of Obviousness Type Double Patenting

The Examiner rejected Claims 1-27 under the Doctrine of Obviousness Type Double Patenting over Claims 1-15 of Lévesque *et al.* (U.S. Patent No. 6,087,168), in view of Chang *et al.* (Development [Eng] 126[15]:3347-57 [1999], abstract). The Examiner stated that a timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. The grounds of rejection included the following:

> **The instant claims 1-48 differ from the claims of '168 by claiming the step of adding an amount of an antagonist of bone morphogenetic protein (BMW) to antagonize endogenous BMP signal transduction activity in the methods of making transdifferentiated epidermal basal cells. The claims specify wherein the antagonist of bone morphogenetic protein (BMP) is fetuin, noggin, chordin, gremlin or follistatin.**
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> **Chang et al. teach "ectodermal cell fates are determined by antagonistic interaction between the BMP subfamily of TGF-(beta) ligands and the organizer-specific secreted factors (e.g. noggin, chordin and follistatin). Inhibition of BMP function by these factors can convert cells from an epidermal to a neural cell fate. In this study, we report that GDF6, a new member of the Xenopus TGF-(beta) family, can function in antagonistic interaction with neural inducers. GDF6 induces epidermis and inhibits neural tissue in dissociated cells, and this activity is blocked by the presence of noggin.... In addition, BMP and GDF6 heterodimers may play an important role in vivo to regulate cell fate determination and patterning." One of ordinary skill in the art would thus have been motivated to downregulate BMP in methods of converting cells from an epidermal to a neural cell fate as taught by Chang et al. for instance.**
>
> **Furthermore, the open comprising language of the claims of '168 broadly encompasses the instant claims 1-48.**

In response, Applicant is willing to file a terminal disclaimer in the event any claims of the above-captioned application be found allowable.

B. Rejections under 35 U.S.C. § 112, first paragraph

(1) Claims 17-39 and 43-48 were rejected under 35 U.S.C. § 112, first paragraph. The Examiner based the rejection on the assertion that:

> **. . . Claims 17-39 and 43-48 are drawn to a breadth of transdifferentiated cells, kits comprising said cells and cell cultures having a broad scope of morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal or glial cell.**
>
> **The specification as filed teaches that human adult skin was cultured and transfected with pRcCMVneo vectors containing B-gal, NeuroD1, NeuroD2, hASH1, Zicl or hMyTl human genes. The specification teaches in example 3 the design of two antisense oligonucleotides to target human MSX1 and two antisense oligonucleotides to target human HES1. In example 4, the specification teaches the methods for detection of transdifferentiation of the epidermal cells to neural cells as immunohistochemical detection of neurofilament M, neural specific tubulin, neural specific enolase, microtubule associated protein 2, neurofilaments Mix, flial fibrillary acidic protein, and morphological criteria. The specification teaches that cells with neurites longer than three cell diameters (50 microns or longer) and expressing at least one neuronal marker were counted as neurons. Table 1 teaches the results of the transdifferentiation experiments showing that a combination of neurogenic transcription factor expression coupled with decrease in MSX1 and HES1 expression was most effective at establishing transdifferentiation.**
>
> **The claims encompass transdifferentiated neuronal cells made by different methods or having different physiological characteristics as described above. The specification however, only teaches in Table 1 a defined set of cells having some characteristic of a differentiated neuronal cell, the structure of which is not adequately described therein. The specification teaches broadly that different characteristics of neuronal cells were evaluated, but does not specify the uniformity of such characteristics amongst or between the cells having different transcription factors and antisense sequences applied. Therefore, it is not clear to one of skill in the art that the cells taught as differentiated neuronal cells have the structures as claimed or that a representative number of such cells was described by the specification as filed . . .**

First, Applicant brings the Examiner's attention to the fact that the claimed invention does not involve transfection with pRcCMVneo vectors containing B-gal, NeuroD1, NeuroD2, hASH1, Zicl or hMyTl human genes. (See, e.g., Claim 1). The Examiner's reference to the design of MSX1- and HES1-targeting antisense oligonucleotides was more accurately directed to Example 1, not to Example 3. Also, the Examiner's reference to "Example 4", with respect to methods for detection transdifferentiation, was erroneous, since the above-captioned application does not contain an Example 4. Applicant understands that the Examiner may have intended "Example 1" and other places in the specification, e.g., at page 14, line 10 through page 17, line 28.

Second, and contrary to the Examiner's assertion, the specification does reasonably convey to the skilled artisan that Applicants were in possession of the claimed invention. Claims 17-39 are drawn to a "transdifferentiated cell of epidermal origin having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell . . ." Claims 43-48 relate to a kit for transdifferentiating an epidermal

basal cell into a cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell. The specification discloses numerous useful examples of morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell and methods for detecting them. (E.g., Specification, at page 14, line 10 through 17, line 28). By way of example, the specification discloses in detail how neural-specific antigenic markers were detected on the surfaces of transdifferentiated cells (at page 28, line 10 through page 29, line 25). In addition, the specification shows that many transdifferentiated cells expressed processes 50 microns or longer, like the morphological neurites of neuronal cells (page 9, lines 7-11; Figure 1B).

A transdifferentiated cell in accordance with the claimed invention must have at least one morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell, but uniformity of antigenic markers among the individual cells is not an essential element of the claimed cell.

One skilled in the art would know that successful transfection methods typically require a certain statistically significant number of cells. In the instant case, a transfection protocol was used in which epidermal cells were plated in 6-well tissue culture plates at a density of 50,000 cells per well (e.g., specification, at page 26, lines 23-25). The specification as filed (Figures 1B-1C) shows a portion of a microscopic field of treated cells in monolayer– and shows more than a statistically significant number of them (greater than 30), but this is only a representative portion of the 100-300 cells in each of the five to seven fields of immuno-stained cells that were counted (e.g., at page 28, lines 8-9). Processes can be seen in a number of cells in Figures 1B and 1C, and the specification further discloses that 22-27% of them were neurofilament M immunoreactive (Table 1, page 29, lines 24-25).

Because for convenience the data in Table 1 relate to the determination of only one neural-specific antigen (neurofilament M), absolute numbers of successfully transdifferentiated cells that express another of the useful markers would be underestimated. But the relative values provided in Table 1 are sufficient to show one of skill in the art the relative effectiveness of various combinations of antisense oligonucleotides and neurogenic factors in accordance

with the claimed method. The range of effectiveness among the various treatments illustrated in Table 1 extends over at least three orders of magnitude, which indicates that the values in Table 1 reflect real effects on as many as 27% of the treated cells, and possibly more. Thus, Table 1, in conjunction with Figure 1B-1C and other disclosures in the specification, indeed conveys that the Applicants were in possession of the claimed transdifferentiated cells.

The Examiner also stated:

> **. . . Further it is not clear from the specification as filed that the cells taught as having some or several such differentiation markers could be considered differentiated neuronal cells per se. The activation of one or several genes in epidermal cells leading to the transcription of one or more neuronal markers or a single physiological response does not indicate that such modified epidermal cells would necessarily have the function of neuronal cells based on cell acquisition of one or several such morphological features.**
>
> **The claims drawn to kits containing ingredients for differentiation of epidermal cells to neuronal cells are further not adequately described by the specification as filed because the specification does not teach the structures of the cells based on the application of the various kit components.**
>
> **In summary, the claims are drawn to a genus of transdifferentiated neuronal cells having different characteristics yet the specification as filed does not teach the correlation of these characteristics to the methods applied for transdifferentiation of the epidermal cells. Therefore, while the cells may suggest specific neuronal features, they do not show possession of a representative number of such whole, complete, neuronal cells to show possession to one of skill in the art of the genus of differentiated neuronal cells as claimed**

As noted above, Claims 17-38 (and 39 directed to a cell culture) are drawn to a "transdifferentiated cell of epidermal origin having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell . . .", rather than to a neuronal cell *per se*. Thus, Applicant believes that there is no basis for the rejection on this ground.

In particular, Applicants agree with the Examiner that whether a transdifferentiated cell is a "neuronal cell per se" is not a clearly defined determination. To one of skill in the art, a "neuron" is defined by several criteria including morphology (e.g., long processes or neurites), expression of a set of neural markers (e.g., neurofilament M, neural-specific tubulin, neural-specific enolase, microtubule associated protein 2, and others), synthesis of neurotransmitters (e.g., dopamine or expression of tyrosine hydroxylase, the key enzyme in dopamine synthesis), and membrane excitability. Generally only a small operative subset of the possible criteria are examined with respect to a particular neuronal population within a given context.

It is known in the art that every neuronal population expresses a specific set of neural markers, neurotransmitters, and receptors, and that as neuronal precursor cells differentiate into other neuronal cell types in response to physiological signals in the microenvironment, the set that is expressed will be different. (E.g., see Exhibit A: D.L. Stemple and N.K. Mahanthappa, *Neural stem cells are blasting off*, Neuron 18:1-4 [1997]; Exhibit B: Y. Renoncourt *et al.*, *Neurons derived in vitro from ES cells express homeoproteins characteristic of motoneurons and interneurons*, Mechanisms of Development 79:185-97 [1998]; Exhibit C: A.J. Kalyani *et al.*, *Spinal cord neuronal precursors generate multiple neuronal phenotypes in culture*, J. Neurosci. 18(19):7856-68 [1998]; copies attached). The transdifferentiated cells of the claimed invention are useful in that, *inter alia*, they can be manipulated, in vitro in the presence of specific exogenously supplied signal molecules, or in vivo within specific microenvironments, into diverse types as defined by the operative criteria. (See, e.g., specification, at page 5, lines 14-29; at page 17, lines 17-25; and at page 18, line 25 through page 19, line 4).

As to Claims 43-48, directed to a kit for transdifferentiating an epidermal basal cell into a cell having one or more morphological, physiological and/or immunological feature(s) of a neural progenitor, neuronal, or glial cell, the relationship between the components of the claimed kit and the characteristics of the claimed transdifferentiated cells are indeed described in the specification in some detail. For example, the role of an antagonist of bone morphogenetic protein (BMP), such as fetuin, noggin, chordin, gremlin, or follistatin, is described in the specification, e.g., at page 2, line 28 through page page 4, line 19; and at page 11, line 7 through page 12, line 11. Figure 2 shows a dose-dependent effect of the BMP antagonist. The role of the antisense oligonucleotide comprising a segment of a human MSX1 gene or HES1 gene (negative regulators of neural differentiation), or a non-human homologous counterpart is described, for example, at page 3, lines 14-24; at page 4, line 28 through page 5, line 13; at page 9, line 26 through page 10, line 4; and page 12, line 12 through page 13, line 20. Induction of neural differentiation, or at least induction of neuron-like characteristics, that occurs in the presence of a retinoid and optionally a neurotrophin, such as BDNF, CNTF,

PDGF, NGF, NT-3, NT-4, or sonic hedgehog is described, for example, at page 13, line 21 through page 14, line 9; at page 16, lines 2-7; and page 17, lines 12-21. In addition, one of skill in the art is familiar with the role of retinoids, such as retinoic acid, in inducing differentiation of some neural populations (e.g., specification, at page 5, lines 9-13; and Exhibit B: Y. Renoncourt *et al.* [1998], at 186, column 2, first complete paragraph; Section 2.3, page187, column 1 through page 189, column 2).

Therefore, Applicant respectfully requests the Examiner to withdraw the rejection of Claims 17-39 and 43-48 on this ground.

(2) Claims 1-48 were rejected under 35 U.S.C. § 112, first paragraph. While the Examiner noted that the specification is enabling for differentiated cells showing some specific neuronal cell features, Claims 1-48 were rejected for the following reasons:

> **. . . Claims 1-48 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for differentiated cells showing some specific neuronal cell features, does not reasonably provide enablement for the scope of methods for making neuronal cells as claimed. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.**
>
> **Claims 1-48 are drawn to methods of transdifferentiating an epidermal cell into a neuronal cells and the cells produced by said process.**
>
> **The specification as filed teaches that human adult skin was cultured and transfected with pRcCMVneo vectors containing B-gal, NeuroD1, NeuroD2, hASH1, Zic1 or hMyT1 human genes. The specification teaches in example 3 the design of two antisense oligonucleotides to target human MSX1 and two antisense oligonucleotides to target human HES1. I In example 4, the specification teaches the methods for detection of transdifferentiation of the epidermal cells to neural cells as immunohistochemical detection of neurofilament M, neural specific tubulin, neural specific enolase, microtubule associated protein 2, neurofilaments Mix, glial fibrillary acidic protein, and morphological criteria. The specification teaches that cells with neurites longer than three cell diameters (50 microns or longer) and expressing at least one neuronal marker were counted as neurons. Table 1 teaches the results of the transdifferentiation experiments showing that a combination of neurogenic transcription factor expression coupled with decrease in MSX1 and HES1 expression was most effective at establishing transdifferentiation.**
>
> **Claims 1 and 5 as written broadly encompasses any differentiated neuronal cell originating as a skin cell where a DNA encoding a neurogenic transcription factor is expressed and an antisense to a negative regulator of neuronal differentiation is expressed. The specification as filed however, only teaches differentiated cells having specific physical or marker characteristics of neuronal cells as a result of specific expression of neuronal transcription factors with and without antisense suppression (see Table 1).**
>
> **This method of differentiation of epidermal cells to neuronal cells recites several key steps for achieving a cell having certain neuronal characteristics, but is unpredictable in the following instances:**
>
> **The epidermal cell obtained from the patient is not specified as to type nor are any other physical characteristics given to determine the susceptibility of the cell to dedifferentiation, further, the specification as filed does not specify how the cells were dedifferentiated . . . Specifically example 1 teaches a calcium-free medium for growth of the skin cell culture, but does not provide guidance for what other cytokines, growth factors or genetic manipulation (claim 2) would be necessary to dedifferentiate the cells.**

First, Applicant brings the Examiner's attention to the fact that the claimed invention does not involve transfection with pRcCMVneo vectors containing B-gal, NeuroD1, NeuroD2, hASH1, Zicl or hMyTl human genes. (See, e.g., Claim 1). The Examiner's reference to the design of MSX1- and HES1-targeting antisense oligonucleotides was more accurately directed to Example 1, not to Example 3. Also, the Examiner's reference to "Example 4", with respect to methods for detection transdifferentiation, was erroneous, since the above-captioned application does not contain an Example 4. Applicant understands that the Examiner may have intended Example 1 and other places in the specification, e.g., at page 14, line 10 through page 17, line 28. Applicant presumes that the Examiner's reference to Claim 5 was actually directed to Claim 17.

Second, Applicant disagrees that the specification lacks enablement for the claimed methods and transdifferentiated cells and cell cultures. For example, the specification, describes the source of the epidermal basal cells, their separation from keratinized cells, and the fact that no further dedifferentiation step is necessary (e.g., at page 10, line 15 through page 11, line 6). It is known that low calcium ion concentration results in the stripping of the keratin-forming upper epidermal layers from the basal cells, which is followed by rapid basal cell proliferation when calcium ion is returned to the dedifferentiated cell population. (E.g., specification, at page 11, lines 1-4; and P.K. Jensen and L. Bolund, *Low Ca^{2+} stripping of differentiating cell layers in human epidermal cultures: an invitro model of epidermal regeneration*, Experimental Cell Research 175:63-73 [1988], copy attached as Exhibit D). As the specification states, at page 11, lines 4-6, "Beyond this, it is not necessary to do a dedifferentiating step with respect to individual epidermal basal cell(s) after they are separated, isolated, or selected from the differentiated keratinized cells."

Example 1 demonstrates how before transfection, the [keratinized] differentiated cell layers were stripped from the basal cells in calcium-free medium and then removed from the culture (at page 26, lines 10-25). Further, the specification demonstrates that the claimed method of transdifferentiating an epidermal basal cell indeed works to transdifferentiate at least some large subset of the cell population (see, e.g., Example 2, Table 1, especially at page 29,

lines 24-25), the identity of which subset is not material to enablement.

Consequently, Applicant respectfully requests the Examiner to withdraw the rejection on this ground.

The Examiner also stated:

> **. . . The specification teaches only expression of certain neurogenic transcription factors in combination with certain antisense oligonucleotides resulting in cells having a phenotypic or neurogenic marker expression . . .**
>
> **. . . In the case of antisense oligonucleotides to negative regulators of neuronal differentiation, there is a high level of unpredictability known in the antisense art for design of antisense molecules to known target genes (see Branch). The factors considered unpredictable are (1) delivery and stability of the antisense molecule, (2) availability of the intended target site, and (3) effective antisense action marked by a decrease in the intended target expression. The specification only teaches antisense to two genes, human MSX1 and human HES1. Therefore it would require "trial and error" experimentation to design antisense molecules to other negative regulators of neuronal differentiation as claimed.**
>
> **The lack of guidance in the specification as filed for these factors would therefore require "trial and error" experimentation beyond which is taught by the specification as filed. Therefore, it would require undue experimentation to practice the invention as claimed . . .**
>
> **. . . the antisense inhibition of the transdifferentiated cells would alone present a high level of unpredictability. There is a high level of unpredictability known in the antisense art for therapeutic, in vivo (whole organism) applications. The factors considered barriers to successful delivery of antisense delivery to the organism are: (1) penetration of the plasma membrane of the target cells to reach the target site in the cytoplasm or nucleus, (2) withstanding enzymatic degradation, and (3) the ability to find and bind the target site and simultaneously avoid non-specific binding (see Branch). Despite the synthesis of more resilient, nuclease resistant, oligonucleotide backbones and isolated successes with antisense therapy in vivo, the majority of designed antisense molecules still face the challenge of successful entry and localization to the intended target and further such that antisense and other effects can routinely be obtained. Flanagan teaches, "oligonucleotides (in vivo) are not distributed and internalized equally among organs and tissues .... Unfortunately, therapeutically important sites such as solid tumors contain very little oligonucleotide following intravenous injections in animals (page 51, column 2)."**
>
> **Specifically, in vitro results with one antisense molecule are not predictive of in vivo (whole organism) success. In vitro, antisense specificity to its target may be manipulated by "raising the temperature or changing the ionic strength, manipulations that are commonly used to reduce background binding in nucleic acid hybridization experiments." (Branch, p. 48) Discovery of antisense molecules with "enhanced specificity" in vivo requires further experimentation for which no guidance is taught in the specification. Note Branch who teaches the state of the art for designing an antisense which inhibits a target in vivo: it "is very difficult to predict what portions of an RNA molecule will be accessible in vivo, effective antisense molecules must be found empirically by screening a large number of candidates for their ability to act inside cells (Branch, p.49)." And in the instant case, the therapeutic claims read broadly on administration of an antisense inhibitor in any transdifferentiated cell to a whole organism included. While the specification teaches cell culture inhibition, no evidence of successful in vivo (whole organism) antisense inhibition has been shown, nor do the culture examples correlate with whole organism delivery.**
>
> **One of skill in the art would not accept on its face the successful delivery of the disclosed antisense molecules in vivo and further, treatment effects, in view of the lack of guidance in the specification and the unpredictability in the art. Neither the specification nor technology today teach general guidelines for successful delivery or treatment effects of antisense molecules in whole organisms. Specifically the specification does not teach (1) stability of the antisense molecule in vivo, (2)effective delivery to the whole organism and specificity to the target tissues, (3) dosage and toxicity, nor (4) entry of molecule into cell and effective action therein marked by visualization of the desired treatment effects. These key factors are those found to be highly unpredictable in the art as discussed supra. The lack of guidance in the specification as filed for these factors would therefore require "trial and error" experimentation beyond which is taught by the specification as filed.**
>
> **Therefore, it would require undue experimentation to practice the invention as claimed . . .**

Contrary to the Examiner's assertion that ". . . it would require 'trial and error' experimentation to design antisense molecules to other negative regulators of neuronal differentiation as claimed," no antisense oligonucleotides targeting *other* negative regulators are claimed in the method of Claim 1. Applicant believes that Claim 1, does *not* imply that

antisense oligonucleotides directed to suppressing negative regulators *other* than MSX1 and HES1 are used in the method, since only MSX1- and/or HES1-targeting antisense oligonucleotides were recited in paragraph (c) of Claim 1, as originally filed. Also, paragraph (c) (as amended) recites "growing the cells in the presence of at least one antisense oligonucleotide comprising a segment of a human MSX1 gene and/or human HES1 gene, or homologous non-human counterpart of either of these, in an amount sufficient to suppress the expression of functional gene product of *MSX1* and/or *HES1* . . ."

In addition, the Examiner's remarks, and the cited Flanagan *et al.* and Branch references, regarding the unpredictability of antisense therapy *in vivo* are irrelevant and clearly not properly directed to the claims as filed, which do not contain limitations concerning transfection. The claimed invention does not relate to in vivo delivery of antisense molecules or any other variety of in vivo gene therapy. Applicant's disclosures pertaining to *in vitro* delivery to epidermal basal cells of oligonucleotides targeting MSX1 and HES1 are clearly enabling (see, e.g., Examples 1-3).

Consequently, Applicant respectfully requests the Examiner to withdraw the rejection on this ground.

The Examiner further stated:

> **. . . In regards to the screening and diagnostic claims, the specification as filed teaches only prophetically methods of application for screening and therapeutic applications of the transdifferentiated cells of claims 1 or 5.**
>
> **The specification as filed therefore does not provide any guidance for the transplantation of transdifferentiated cells/tissues into a patient nor does it provide any evidence of the cells ability to form functional connections and operate as true neurons either in vitro or in a whole organism.**
>
> **The factors considered unpredictable for such treatment would include the ability of the cells to retain the transdifferentiated state, for example. . .**
>
> **. . . Further, no guidance is taught by way of example for assaying the effect of the potential new drugs on a physiological or molecular biological property of said transdifferentiated neuronal cells. The only physiological or molecular biological properties taught are those characteristics applied to determination of the neuronal status of the transdifferentiated cells such as morphological occurance of a neurite and immunological expression of neuronal antigens.**

In order to meet her burden in rejecting the claims as lacking enablement, the Examiner is required to provide a reasonable basis to question the enablement provided for the claimed invention. (*In re Wright*, 999 F.2d 1557, 27 USPQ2d 1510 [Fed. Cir. 1993]). This burden includes providing sufficient reasons for doubting any assertions in the specification as to the

scope of the enablement. Only if the Examiner meets her burden does the burden shift to Applicant to provide suitable proofs indicating that the specification is enabling. In addition, an Applicant need not have actually reduced the invention to practice prior to filing. (*Gould v. Quigg*, 822 F.2d 1074, 1078 [Fed. Cir. 1987]). Prophetic examples based on predicted results are allowed for purposes of compliance with the requirements of 35 U.S.C. § 112, first paragraph. (MPEP 2164.02).

The Examiner's assertion, that the specifications provide no guidance for the transplantation of transdifferentiated cells/tissues into a patient nor evidence for the cells' ability to form functional connections and operate as true neurons . . . in a *whole organism*, is irrelevant to the claimed *in vitro* methods of using cells transdifferentiated from epidermal basal cells to identify a novel nerve growth factor (e.g., Claim 40), or to identify a potential chemotherapeutic agent (e.g., Claim 41), or to screen a potential chemotherapeutic agent to treat a nervous system disorder of genetic origin (e.g., Claim 42). The claimed invention does not relate to transplanted cells.

As to whether the cells' ability to form functional connections and operate as true neurons in vitro, this also does not bear on the enablement provided by the specification, as stated in the specification, e.g., at page 25, lines 3-11. In accordance with the methods of Claims 40-42, the transdifferentiated cells are assayed in vitro to determine whether there is an effect of a potential nerve growth factor or chemotherapeutic agent on a physiological or molecular biological property of the transdifferentiated cells. The use of transdifferentiated epidermal basal cells bypasses the difficulties in isolating and culturing neuronal cell types from the brain or human fetal tissue. (See, specification, e.g., at page 6, lines 26-30). As noted above, the specification teaches a method of transdifferentiation of epidermal basal cells and discloses that the further course of development of the transdifferentiated cells depends on the in situ environmental cues to which they are exposed. Thus, the transdifferentiated cells can be manipulated to express a set of properties (e.g., morphological or antigenic) that is characteristic of certain populations of neurons. The transdifferentiated cells may or may not express all the biochemical, morphological, physiological and functional characteristics of a

given neuronal or glial cell population. For example, they may or may not form functional interneuronal connections. But regardless, they are at least useful simulations of neurons or glial cells for screening or isolating promising new drugs or neural growth factors. Once the potential of a chemical agent is identified by the claimed methods, then, of course, further research can be done to verify its actual effect on particular cell populations of the nervous system and ascertain its clinical usefulness. (See, specification, e.g., at page 23, lines 12-16; and especially at page 25, lines 3-11). Therefore, the methods claimed in Claims 40-42 are enabled, regardless of whether interneuronal connections are formed in vitro.

Also contrary to the Examiner's assertion, the specification as originally filed indeed provides guidance for assaying the effect of the potential new drugs (e.g., Claims 41-42), or novel nerve growth factors (e.g., Claim 40), on a physiological or molecular biological property of the transdifferentiated cells, other than the morphological occurrence of a neurite and immunological expression of neuronal antigens. As can be seen (e.g., specification, at page 23, line 3 through page 25, line 11), the useful assay techniques described for detecting effects of potential chemotherapeutic agents [or novel growth factors] on particular cell types include those based on electrophysiological characteristics (e.g., patch clamp or intracellular electrophysiological recording [e.g., page 24, lines 2-4]), gene expression profiles, neurotransmitter profiles, cytoskeletal organization, organization of ion channels and receptors, and effects on cell survival (e.g., page 24, lines 2-6; and page 24, line 28 through page 25, line 2), which are detectable by methods known to those skilled in the arts of molecular biology and cell culture.

The Examiner has failed to meet the burden of providing sufficient reasons to doubt the assertions in the specification. Therefore, Applicants respectfully request that the Examiner withdraw the rejection of Claims 1-48 on this ground.

CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is

now ready for allowance. Early notice to that effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney at (213) 896-6665.

Respectfully submitted,
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